

N-Acetyl-Cysteine Supplementation does not Alter the Erythropoietin Response in Trained
Endurance Athletes in Acute Hypoxia

Tyler J Noble

Submitted to the faculty of the university graduate school in partial fulfillment of the
requirements for the degree.

Master of Science
Indiana University
School of Public Health

Accepted by the Graduate Faculty, Indiana University,
in partial fulfillment of the requirements for the degree of Master of Science

Master's Thesis Committee

Robert F. Chapman, PhD

Chairperson

Timothy D. Mickleborough, PhD

Joel M. Stager, PhD

Copyright © 2021
Tyler J Noble

Abstract

To assess the potential effect of N-Acetyl-Cysteine (NAC) supplementation on the magnitude of EPO response with acute exposure to hypoxia in trained endurance athletes, 10 trained male endurance athletes engaged in a placebo-controlled crossover design, featuring two conditions: a placebo (PLA) condition and an NAC condition. Each condition featured a day of baseline testing followed by an 1800 mg/day supplementation period lasting eight days and, finally, a six-hour acute hypoxic exposure ($\text{FIO}_2 = 15.8\%$, $\sim 2500\text{m}$). Subject serum EPO (EPO), total hemoglobin mass (Hbmass), and hematocrit (Hct) measurements were recorded during the baseline visit of each condition in addition to immediately prior to hypoxic exposure in order to evaluate changes in subject hematological metrics in response to each supplementation condition. Additionally, EPO was measured every two hours during hypoxic exposure to evaluate the EPO response in each condition. Eight days of NAC supplementation prior to acute hypoxic exposure did not significantly alter the EPO (IU/L) response between baseline and pre hypoxic exposure (10.4 ± 2.9 vs. 9.9 ± 1.5 ; $p=0.52$) when compared with the PLA condition (10.9 ± 2.5 vs. 10.2 ± 2.0 ; $p=0.23$). Additionally, no significant changes were seen in Hbmass (g/kg) in the NAC condition (15.75 ± 0.97 vs 16.01 ± 0.68 ; $p=0.58$) when compared to the PLA condition (15.58 ± 0.72 vs 15.84 ± 0.97 ; $p=0.24$). Finally, no significant changes were seen in hematocrit (%) in the NAC condition (46.2 ± 1.6 vs 46.6 ± 1.9 ; $p=0.57$) when compared to the PLA condition (45.8 ± 2.7 vs 46.4 ± 2.2 ; $p=0.23$). EPO increased at each time point after tent entry in both conditions but the magnitude of increase ($\text{NAC} \Delta 32.3 \pm 23.6\%$ vs $\text{PLA} \Delta 31.4 \pm 20.9\%$) was not significantly different between conditions. NAC supplementation prior to acute hypoxic exposure has no significant impact on EPO concentration in trained endurance athletes. The large variation in intra-subject EPO response to hypoxia between conditions may have concealed any impact that NAC supplementation had on EPO response.

TABLE OF CONTENTS

CHAPTER 1	1
INTRODUCTION	1
Statement of the Problem	3
Purpose of the Study	3
Significance of the Study.....	3
Delimitations.....	4
Limitations	4
Assumptions	4
Specific Aims and Hypotheses	4
Definition of Terms	5
CHAPTER 2	6
REVIEW OF LITERATURE.....	6
Introduction.....	6
Approaches to hypoxic training.....	8
HIF-1 α and its role in erythropoiesis.....	9
NAC as a HIF-1 α stabilizer.....	12
Conclusion and rationale for the study.....	15
CHAPTER 3	17
EXPERIMENTAL PROCEDURES	17
Selection of Subjects	17
Study Design	17
Supplementation.....	18

Hypoxic Exposure	19
Specific Measures	19
Height and Weight.....	19
Body Composition.....	20
Blood Sampling.....	20
Blood Processing.....	20
Total Hemoglobin Mass.....	21
Arterial Oxyhemoglobin Saturation.....	21
Treatment of Data	21
Chapter 4	23
RESULTS	23
Chapter 5.....	26
REFERENCES	29
CV.....	

CHAPTER 1

INTRODUCTION

Since the mid-1960s, elite endurance athletes have attempted to enhance exercise performance at sea level through the use of chronic hypoxic exposure. As a result, the validity and efficacy of manipulating hypoxic exposure to enhance performance has been a subject frequently studied within the scientific community. Research in this field has highlighted the many physiological adaptations to hypoxic exposure (Chapman, Stray-Gundersen, and Levine 1998; B. Levine and Stray-Gundersen 1992; Naeije 2010; Wilber 2007). Perhaps the most important adaptation, in regards to endurance performance, is the marked expansion in hemoglobin mass associated with chronic exposure to hypoxia (Chapman et al. 2014; Chapman, Stray-Gundersen, and Levine 1998; B. D. Levine and Stray-Gundersen 1997; B. Levine and Stray-Gundersen 1992; Wilber 2007). This hematological response enhances the oxygen carrying capacity of the blood; which is widely considered to be the primary performance enhancing outcome of chronic altitude training by endurance athletes (Chapman et al. 2014; Chapman, Stray-Gundersen, and Levine 1998; B. Levine and Stray-Gundersen 1992).

Investigations into the optimization of the hematological profile in response to hypoxia have largely centered on enhancing the production of the protein/hormone erythropoietin (EPO), which serves as the primary control for the expansion of red blood cells and hemoglobin mass (Bauer 1988; Eckardt 1989; Fisher 1983). EPO production occurs within the adrenal cortex and is mediated by a number of factors, most notably, the stabilization of Hypoxia Inducible Transcription Factor (HIF-1 α) (C Bauer 1988; Christian Bauer and Kurtz 1989; Fisher 1983).

HIF-1 α stabilizes in response to a mismatch between oxygen demand and oxygen availability, as perceived by oxygen sensing mechanisms located within the body (Harms and

Stager 1995; Huang et al. 1996). The rapid degradation period of stabilized HIF-1 α under normoxic conditions presents a challenge to athletes attempting to enhance EPO production (Lee et al. 2004). Ultimately, since the EPO response to hypoxia is proportional to the level of hypoxic stress, athletes could simply maximize EPO production by living at higher elevations (Stager 1995; Huang et al. 1996). However, living at elevations above 2500 meters is associated with acute and chronic acclimatization processes which may be detrimental to both health and training status (Bärtsch and Saltin 2008; Hackett and Roach 2001; Roach et al. 2000; Schneider et al. 2002). Furthermore, endurance athletes living above 2500 meters may struggle to train at the intensities necessary to maintain sea level performance (Chapman et al. 2014; Chapman, Stray-Gundersen, and Levine 1998; B. D. Levine and Stray-Gundersen 1997). It stands to reason then, that elite endurance athletes engaging in hypoxic training should explore methods of enhancing the EPO response beyond merely attempting to live at increasingly greater elevations. Select dietary interventions in concert with exposure to moderate hypoxia (2000-2500m) may be a simple and relatively easy method to achieve this outcome and, subsequently, enhance performance.

Research into the antioxidant, N-Acetyl-Cysteine (NAC) has suggested that the readily available, over-the-counter cysteine supplement, works as a mild stabilizer of HIF-1 α (Hildebrandt et al. 2002; A Zembron-Lacny et al. 2010). The cysteine sulfanyl group has been shown to alter the thiol content of the blood, primally through the generation of glutathione with NAC supplementation, thereby altering the oxygen sensing ability of the chemoreceptors in the body (Hildebrandt et al. 2002; Momeni et al. 2011; Tajima et al. 2009). This is thought to stabilize HIF-1 α and, subsequently, upregulate EPO production (Hildebrandt et al. 2002; A Zembron-Lacny et al. 2010). To date investigations into NAC and its potential role in the EPO

production have been limited to diseased or untrained populations (Hildebrandt et al. 2002; Momeni et al. 2011; Agnieszka Zembron-Lacny et al. 2009). Further, studies into the effect of anti-oxidant supplementation on EPO response to hypoxic environments have not included trained athletes (Hildebrandt et al. 2002; Momeni et al. 2011; Agnieszka Zembron-Lacny et al. 2009).

Statement of the Problem

Due to an individualized physiological response, trained endurance athletes may struggle to optimize desired hematological adaptations derived from exposure to hypoxia.

Purpose of the study

The purpose of this study is to explore the potential augmentative effect of the antioxidant N-Acetyl-Cysteine on the magnitude of EPO response with acute exposure to hypoxia in elite endurance athletes.

Significance of the study

A large portion of trained endurance athletes utilize hypoxic exposure as a means to augment hematological markers that impact performance (Chapman et al. 2014; Chapman, Stray-Gundersen, and Levine 1998). As a result of the individualized response to hypoxia, many endurance athletes struggle to optimize hematological adaptations to hypoxic exposure (Chapman, Stray-Gundersen, and Levine 1998; Harms and Stager 1995). If dietary interventions, such as NAC supplementation, are able to augment the EPO response, elite athletes may be able to optimize hematological adaptation and thereby improve athletic performance.

Delimitations

1. Subjects selected for this study will be highly trained endurance athletes.
2. Medical grade assays will be used in an effort to ensure a quality measurement of blood markers.
3. The study will rely on the use of randomized, repeated measures, cross-over study design where subjects will serve as their own control group in an attempt to control for an individualized response to hypoxia.

Limitations

1. The sample size of the study will be small and may impact the any statistical conclusions drawn from the results.
2. The fitness level between subjects may vary and affect the ability to generalize findings to elite athletes.

Assumptions

1. We assume that all subjects selected to participate in this study are highly trained endurance athletes.
2. We assume that all subjects are healthy and free from any neuroendocrine limitation that might constrain their response to hypoxia.
3. We assume that exposure to both normobaric and hypobaric hypoxia result in identical physiological responses.

Specific Aim and Hypothesis

Specific Aim: To determine whether or not prior NAC supplementation augments the EPO response to hypoxia in highly trained endurance runners

Research hypothesis for specific aim:

Eight days of NAC supplementation prior to hypoxic exposure will augment the magnitude of the EPO response to 6 hours of acute hypoxic exposure beyond placebo supplementation in elite endurance athletes.

Definition of Terms

Erythropoietin (EPO): The protein/hormone responsible for upregulating the production of hemoglobin mass and red blood cells.

Anti-oxidant: A substance that remedies oxidative stress and neutralizes free radicals.

N-Acetyl-Cysteine (NAC): A commercially available, anti-oxidant supplement.

Hypoxic Inducible Transcription Factor (HIF-1 α): The protein responsible for upregulating the production of EPO when stabilized.

Hematological Adaptations: Changes in the blood markers as a result of a given stimuli.

Reactive Oxygen Species (ROS): Reactive molecules and free radicals derived from molecular oxygen.

Hypoxia: Deficiency in atmospheric oxygen; 15.8% Po₂

Trained: Endurance athletes averaging over 300 minutes of aerobic activity weekly

CHAPTER 2

REVIEW OF LITERATURE

Introduction

Hypoxic training has become a common practice amongst elite endurance athletes hoping to enhance endurance sport performance(Wilber 2007). The primary hematological adaptation to hypoxia that is associated with endurance sport performance, increased red cell and hemoglobin mass, is reliant upon the stability of HIF-1 α (Fisher 1983; Koh, Spivak-Kroizman, and Powis 2008; B. Levine and Stray-Gundersen 1992; Naeije 2010). The induction status of HIF-1 α serves as the signal for the production of EPO within the renal cortex, which initiates the erythropoietic process(Christian Bauer and Kurtz 1989; Fisher 1983; Naeije 2010). With that being said, the relative instability of HIF-1 α in concert with the highly variable and individualized response to hypoxia presents a problem for athletes when prescribing guidelines for hypoxic training(Chapman, Stray-Gundersen, and Levine 1998; Wilber 2007). HIF-1 α stabilization is mediated by a number of oxygen dependent process and thus, the erythropoietic response to hypoxia is proportional to the level of perceived hypoxic stress (Eckardt 1989; Koh, Spivak-Kroizman, and Powis 2008). There are, however, functional limitations in maximizing erythropoiesis by maximizing hypoxic stress and athletes should be made aware of potential adverse consequences associated with living and training at elevations greater than 2500 meters (Bärtsch and Saltin 2008; Buskirk et al. 1967; Harms and Stager 1995; B. Levine and Stray-Gundersen 1992; Roach et al. 2000; Schneider et al. 2002). Due to these contraindications, athletes may want to seek interventional methods designed augment the erythropoietic response to hypoxia at elevations below 2500 meters(Chapman et al. 2014; B. Levine and Stray-Gundersen 1992; B. D. Levine and Stray-Gundersen 2006; Wilber 2007). It is then the purpose

of this review to explore the validity and efficacy of two dietary interventions which may augment the erythropoietic response to a fixed level of moderate hypoxia. Relevant literature regarding the following topics will be carefully considered: (1) A brief review of hypoxic training models (2) HIF-1 α and its role in erythropoiesis (3) N-Acetyl-Cysteine as a mild HIF-1 α stabilizer.

Approaches to Hypoxic Training

In the mid-1960s, athletes and coaches started to utilize hypoxic training as means to improve performance in hypoxia. Over time, this focus has morphed into utilizing hypoxic training as a means to enhance normoxic performance and has grown to become common practice amongst endurance athletes as a means to enhance performance in recent years. As a result, the physiological response to hypoxia as it relates to endurance sport performance has been a heavily researched topic amongst sport scientists (Chapman, Stray-Gundersen, and Levine 1998; B. Levine and Stray-Gundersen 1992; Wilber 2007). This expansion of research into hypoxic training has yielded many training models, two of which will be compared for the purposes of this review; the live high, train high (LHTH) and live high, train low (LHTL) models (Wilber 2007). The contradictory nature of these models has led to much debate within the scientific community as to the best practice protocol for hypoxic training to improve endurance performance (B. Levine and Stray-Gundersen 1992; Wilber 2007). While it is widely accepted that the hematological adaptations that occur in response to hypoxia are beneficial for performance, evidence has suggested that impaired training during periods of hypoxic exposure can have a negative impact on performance (Buskirk et al. 1967; B. D. Levine and Stray-Gundersen 1997; Naeije 2010; Niess et al. 2003; Roach et al. 2000; Wilber 2007).

The LHTH model of hypoxic training is considered to be the original hypoxic training protocol and is characterized by long periods of living and training at moderate to high elevations (1500-4000m)(Wilber 2007). Consensus on the effectiveness of LHTH models is lacking, due to a number of study design issues such as group training effects, training camp effects, fitness prior to the start of altitude training, iron status, and adequate (Duke, Chapman, and Levine 2012). However, part of any lack of improvement after LHTH is likely the result of an inability to match sea level training intensities and skeletal muscle oxygen flux at moderate elevations (2500m) (Buskirk et al. 1967; Chapman et al. 2014; Harms and Stager 1995; B. D. Levine and Stray-Gundersen 1997; Niess et al. 2003; Roach et al. 2000). In addition to training impairment, there is a growing body of evidence highlighting the contraindicated health effects to living and training above moderate to high elevations (Bärtsch and Saltin 2008; Hackett and Roach 2001; Roach et al. 2000; Schneider et al. 2002). Chief among these is acute mountain sickness, which has been experienced by elite athletes living and training at elevations as low as 2500m (Bärtsch and Saltin 2008; Hackett and Roach 2001; Roach et al. 2000; Schneider et al. 2002). Furthermore, the high intensity training needed to produce elite level performance may exacerbate negative health outcomes (Hackett and Roach 2001; Roach et al. 2000).

The live-high, train-low (LHTL) approach to altitude training offers a potential solution, as athletes are able to optimize hematological adaptations by living at moderate elevations while maintaining the necessary training intensities and oxygen uptakes by training at lower elevations (Chapman et al. 2014; B. Levine and Stray-Gundersen 1992; B. D. Levine and Stray-Gundersen 1997; Wehrlin et al. 2006; Wilber 2007). The LHTL model has been shown in a number of studies to have a positive impact on both hematological and performance outcomes(Chapman et al. 2014; B. Levine and Stray-Gundersen 1992; B. D. Levine and Stray-Gundersen 1997;

Wehrlin et al. 2006; Wilber 2007). With that being said, due to the highly variable and individualized physiological response to hypoxia, limitations to the overall effectiveness of current LHTL model are likely to exist (Chapman, Stray-Gundersen, and Levine 1998; B. Levine and Stray-Gundersen 1992). While the physiological response to hypoxia is proportional to level of perceived hypoxic stress, contraindications experienced by living and training above 2500m may negate any hematological benefit gained from simply increasing hypoxic stress (Bärtsch and Saltin 2008; Buskirk et al. 1967; Eckardt et al. 1989; Roach et al. 2000; Wilber 2007). Athletes hoping to maximize the hematological and performance outcomes in response to hypoxic training may benefit from interventions designed to augment the physiological response to hypoxia without risking the previously noted contraindications. Such interventions could be optimized if there was greater understanding of the manipulate factors associated with the production of erythropoietin and its transcriptional controller HIF-1 α .

HIF - 1 α and its role in erythropoiesis:

HIF-1 α serves as the master control of cellular hypoxic response and is responsible for the transcription of over 100 genes required for hypoxic adaptation including the upregulation of EPO production (Koh, Spivak-Kroizman, and Powis 2008; Tajima et al. 2009). In regards to endurance sport performance, the most important of these outcomes is the upregulation of EPO production; which occurs within the renal cortex (Chapman, Stray-Gundersen, and Levine 1998; Fisher 1983; B. Levine and Stray-Gundersen 1992; Naeije 2010; Wilber 2007). The protein/hormone EPO serves to signal the expansion of both hemoglobin and red cell mass. This works to enhance the oxygen carrying capacity of the blood, which is widely accepted as the most important factor in aerobic performance (Fisher 1983; B. D. Levine and Stray-Gundersen 2006). Furthermore, the magnitude of the physiological response triggered by HIF-1 α

stabilization has been proven to be directly proportional to the amount of hypoxic stress placed upon it (Eckardt et al. 1989). As a result, endurance athletes training in hypoxia have indirectly sought to maximize the length and intensity of HIF-1 α stabilization in an effort to optimize the physiological adaptations to hypoxia (Chapman et al. 2014; B. D. Levine and Stray-Gundersen 2006).

The rapid degradation and relatively short half-life of activated HIF-1 α presents a challenge to these athletes (Koh, Spivak-Kroizman, and Powis 2008; Lee et al. 2004; B. D. Levine and Stray-Gundersen 2006; Wilber 2007). HIF-1 α stabilization is known to be mediated by a number of oxygen dependent pathways and thus relies on the ability of cells to sense changes in rapidly changing oxygen levels (Christian Bauer and Kurtz 1989; Koh, Spivak-Kroizman, and Powis 2008; Lee et al. 2004). Perhaps the most notable of these pathways involves the ubiquitin degradation by the Von Hippel Lindau (pVHL) protein (Chandel et al. 2000; Jaakkola et al. 2001; Koh, Spivak-Kroizman, and Powis 2008; Li et al. 2005; Ohh et al. 2000). Under normoxic conditions, prolyl hydroxylases (PHD1, PHD2, and PHD3) work to hydroxylate HIF-1 α , and this results in the binding of pVHL to the HIF-1 α oxygen-dependent domain (ODD) (Chandel et al. 2000; Jaakkola et al. 2001; Koh, Spivak-Kroizman, and Powis 2008; Li et al. 2005; Ohh et al. 2000). Once bound to pVHL, this complex is targeted for destruction via the ubiquitin pathway in the proteasome (Jaakkola et al. 2001; Koh, Spivak-Kroizman, and Powis 2008; Li et al. 2005; Ohh et al. 2000). Recent evidence has suggested that prolyl hydroxylase activity may be dependent upon reactive oxygen species (ROS) released from mitochondrial complex III during aerobic metabolism for oxygen sensing and, subsequently, HIF-1 α hydroxylation (Chandel et al. 2000; Jaakkola et al. 2001; Koh, Spivak-Kroizman, and Powis 2008; Simon 2006). Conversely, in hypoxic conditions, PHD activity is inhibited; perhaps by a shift towards a higher reliance

upon anaerobic metabolism at the cellular level(Chandel et al. 2000; Koh, Spivak-Kroizman, and Powis 2008; Li et al. 2005; Ohh et al. 2000). This creates a blunted ROS response from mitochondrial complex III, preventing pVHL binding to HIF-1 α and resulting in HIF-1 α stabilization (Jaakkola et al. 2001; Koh, Spivak-Kroizman, and Powis 2008; Ohh et al. 2000). Upon stabilization, HIF-1 α dimerizes with HIF-1 β and is translocated to the nucleus, stimulating DNA transcription and upregulation of EPO production via REDOX sensitive pathways (Huang et al. 1996; Koh, Spivak-Kroizman, and Powis 2008; Tajima et al. 2009). The interaction between these outcomes appear plays an important role in determining the hematological adaptations in response to hypoxia (Koh, Spivak-Kroizman, and Powis 2008; Tajima et al. 2009).

The upregulation of EPO production within the renal cortex as the result of HIF-1 α induction works to stimulate the formation of erythrocytes within the bone marrow (C Bauer 1988; Christian Bauer and Kurtz 1989; Naeije 2010). The magnitude of the EPO response is proportional to perceived hypoxic stress and peaks around 30 hours in response to prolonged hypoxia (Eckardt et al. 1989; Garvican et al. 2012; Tajima et al. 2009). The erythropoietic process, however, has been shown to take up to 7-10 days while hypoxic training studies have shown appreciable gains in both red cell and hemoglobin mass only after 3-4 weeks of hypoxic exposure (Chapman et al. 2014; B. D. Levine and Stray-Gundersen 2006; Naeije 2010; Wilber 2007). Thus, to achieve optimal hematological and performance outcomes, athletes should seek to maximize the EPO response to hypoxia.

The initial magnitude of EPO production in response to hypoxia dictates the intensity of the hematological, and subsequent, performance response (Eckardt et al. 1989; B. Levine and Stray-Gundersen 1992; Wilber 2007). Since adverse effects to living at elevations above 2500 meters are likely to mitigate the performance benefits of hypoxic training in areas of high

hypoxic stress, a secondary approach might be to maximize any potential benefits through nutritional supplements directed at the underlying biochemical mechanisms. (2000-2500m) (Bärtsch and Saltin 2008; Buskirk et al. 1967; Hackett and Roach 2001; Harms and Stager 1995; Niess et al. 2003; Roach et al. 2000; Schneider et al. 2002).

NAC as a HIF-1 α stabilizer

Research into the antioxidant, N-Acetyl-Cysteine (NAC) has suggested that the over-the-counter supplement works as a mild stabilizer of HIF-1 α (Hildebrandt et al. 2002; Agnieszka Zembron-Lacny et al. 2009; A Zembron-Lacny et al. 2010). The antioxidant properties of NAC work by scavenging ROS and serve as a membrane permeable precursor to glutathione (Chandel et al. 2000; Hildebrandt et al. 2002; Tajima et al. 2009; A Zembron-Lacny et al. 2010). Within the pVHL pathway, NAC supplementation works to scavenge the ROS produced within mitochondrial complexes I & III (Chandel et al. 2000; Hildebrandt et al. 2002; A Zembron-Lacny et al. 2010). The scavenging of mitochondrial ROS inhibits PHD activity and subsequently, the hydroxylation of HIF-1 α (Chandel et al. 2000; Hildebrandt et al. 2002; Koh, Spivak-Kroizman, and Powis 2008; Ohh et al. 2000). This results in the down-regulation of pVHL and the catabolism of HIF-1 α via the ubiquitin proteasome pathway. Therefore, NAC leads to ROS scavenging and stabilization of HIF-1 α (Chandel et al. 2000; Koh, Spivak-Kroizman, and Powis 2008; Li et al. 2005; Ohh et al. 2000).

With NAC's role in the pVHL ubiquitin pathway in mind, there are two key studies which show promise for endurance athletes seeking to optimize hematological factors associated with performance. The first of these studies was the work of Zembron-Lacny et al. (2010) in which the effects of NAC supplementation on blood thiol and plasma EPO concentrations in healthy individuals under normoxic conditions were examined in a placebo-controlled design.

Subjects in the experimental group underwent an 8-day supplementation period in which they consumed 1200 mg/d of NAC. Blood measures collected at the end of the supplementation period within the NAC group displayed a significant rise in both plasma EPO and total thiol concentrations over values collected from the placebo group. Furthermore, the relationship between total thiol and plasma EPO concentrations can be seen in the figure below.

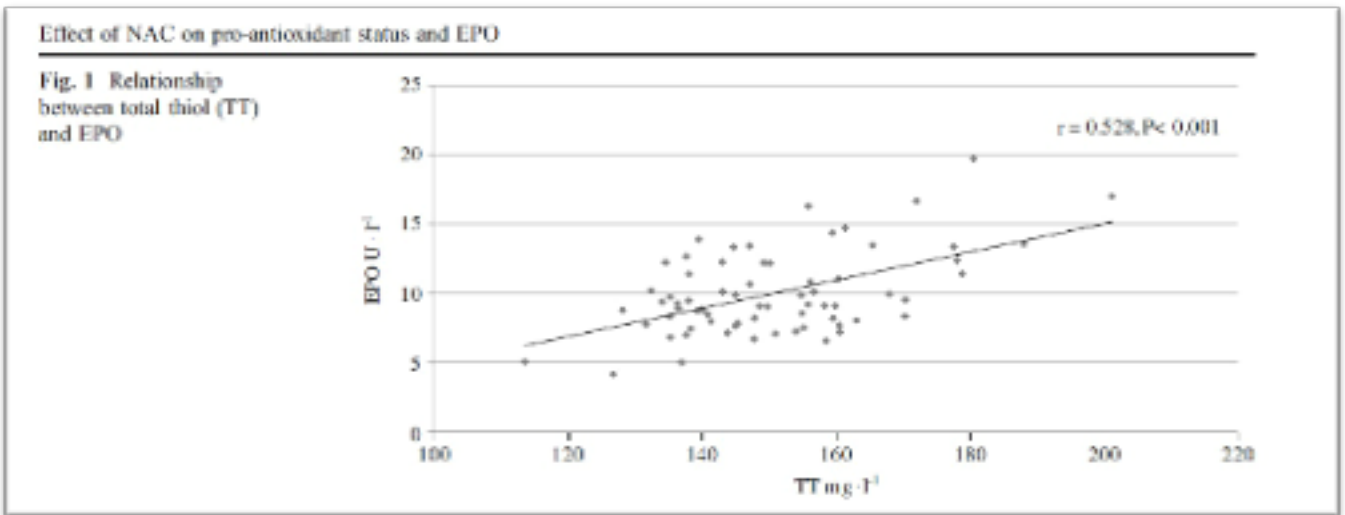


Figure 1. Relationship between total thiols and EPO (erythropoietin) concentration. From Zembron-Lacny et al., 2008

In regard to endurance sport performance, these findings seem to indicate that NAC may be able to enhance EPO production in a manner independent of hypoxic stress; perhaps through the manipulation of plasma thiol content and its known degradation of the pHVL pathway. In theory, a shift in blood thiol content could augment the hematological response to hypoxia without inducing additional hypoxic stress. In the second of these studies, Hildebrandt et al. (2001) sought to assess the hematological effects of a 5-day, placebo controlled, 1800 mg/d NAC supplemental period prior acute exposure (6hrs) to normobaric hypoxia ($\text{FiO}_2 \sim 14\%$). Blood measures of EPO and thiol concentrations were taken on days 1 and 5 of the supplemental period and at 8 different time points during exposure to hypoxia. These samples demonstrated a

significant change in both thiol and EPO concentration between the NAC and control groups as well as pre to post measures. These results can be seen in the figures below:

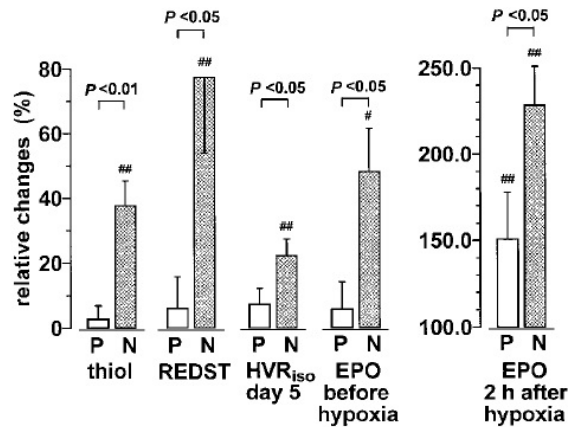
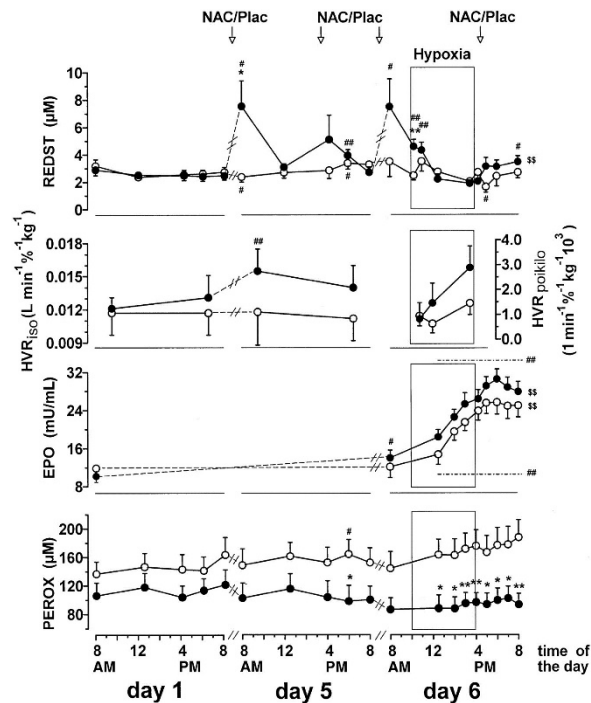


Figure 1. Effect of NAC treatment on the plasma thiol, REDST, HVR, and EPO levels. Relative changes during medication between baseline and terminal examination expressed as percentage of baseline values. With EPO, baseline examination took place on day 1 at 8 AM, and the terminal examination was on day 6 at 8 AM (before hypoxia) or at 6 PM (2 hours after hypoxia), as indicated. In all other cases, the relative changes were computed from the means of all measured values on day 1 and day 5, respectively. Similar data ($P < .05$) were obtained if the HVR data were normalized according to the individual body weight (not shown). Values are mean \pm SEM (P = placebo, $n = 13$; N = NAC, $n = 13$). Significant differences between baseline and terminal values are indicated (## $P < .05$; ### $P < .01$).



Longitudinal changes during NAC treatment and prolonged poikilocapnic hypoxia. Values are means \pm SEM of the data before (day 1) and after 4 days of treatment (day 5) and the data on day 6 before, during, and after a

6-hour exposure to prolonged hypoxia indicated by the windows. The 6-hour exposition to normobaric hypoxia on day 6 started at 10 AM. Three subjects (all placebo) of the total group ($n = 26$) refused to participate in the hypoxic exposition. Significant differences between the NAC group (●) and the placebo group (○) ($*P < .05$; $**P < .01$), significant changes compared with corresponding time points on day 1 ($\#P < .05$; $##P < .01$), and significant posthypoxic changes compared with the last value in hypoxia (day 6, 3:30 PM) ($\$P < .05$; $\$\$P < .01$) are indicated

Taken together, these studies highlight the augmentative effect that NAC has on erythropoiesis under both normoxic and hypoxic conditions. However, both studies are limited in regards to their application to elite athletes as the subjects in the study were described as ‘normal and untrained’ (Hildebrandt et al. 2002; Agnieszka Zembron-Lacny et al. 2009). With that being said, it stands to reason that the consequential shift towards anaerobic metabolism in response to hypoxia that reduces the ROS output from mitochondrial complex III might be augmented further by the ROS scavenging properties of NAC supplementation regardless of training status. (Chandel et al. 2000; Hildebrandt et al. 2002; Koh, Spivak-Kroizman, and Powis 2008; Agnieszka Zembron-Lacny et al. 2009). Additionally, the increase in blood GSH in response to NAC supplementation might elongate the peak EPO response to hypoxia (Ferrara, Gerber, and LeCouter 2003; Hildebrandt et al. 2002; Huang et al. 1996; Tajima et al. 2009). While mild adverse effects have been reported with intravenous supplementation, no adverse effects have been reported with oral NAC supplementation (Rhodes and Braakhuis 2017).

Conclusions and Rationale for the Study

Hypoxic training has been proven to be an effective way to enhance endurance sport performance through the optimization of the hematological profile (B. Levine and Stray-Gundersen 1992; Wehrlin et al. 2006; Wilber 2007). Due to a number of adverse effects of living and training above 2500m, elite endurance athletes may benefit from interventional methods designed to optimize the hematological benefits of living at moderate elevations (2000-2500m) (Chapman et al. 2014; B. D. Levine and Stray-Gundersen 1997; Roach et al. 2000;

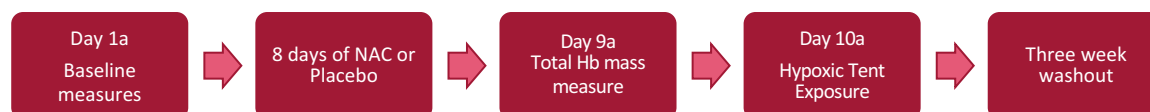
Wilber 2007). The control for EPO production is mediated by the stabilization status of the α -subunit of HIF-1 (Fisher 1983). Supplementation with NAC may work to aid in the stabilization of HIF-1 α through the scavenging of ROS released by mitochondrial complex III (Chandel et al. 2000; Hildebrandt et al. 2002). Furthermore, NAC supplementation is a known, membrane-permeable, precursor to GSH and works to alter the thiol content of the blood (Hildebrandt et al. 2002; Tajima et al. 2009; Agnieszka Zembron-Lacny et al. 2009; Zembron-Lacny et al. 2010). This is believed to enhance the stabilization of HIF-1 α in hypoxia, further augmenting the erythropoietic response.(Ferrara, Gerber, and LeCouter 2003; Huang et al. 1996; Tajima et al. 2009).

CHAPTER 3

EXPERIMENTAL PROCEDURES

Selection of Subjects: 16 well-trained male endurance athletes from Indiana University and the surrounding Bloomington area were recruited for participation in this study. Inclusion criteria was as follows: Subjects were between 18 and 35 years of age, trained a minimum of 5 days a week, and accumulated 300 minutes or more of physical activity weekly. The Indiana University Institutional Review Board approved all protocols and procedures associated with this study and subjects gave informed consent prior to the start of all tests. Subjects were excluded if they indicated a history of respiratory, renal, cardiovascular disease, or smoking. Subjects were also be excluded if they have asthma, known allergies to NAC, previous history of acute mountain sickness, ulcer or other bleeding disorder, impending surgeries, or conditions where NAC is contraindicated – including taking any medications known to have an interaction effect with NAC.

Study Design: Qualifying subjects completed a pseudo-randomized, placebo-controlled cross-over design. Subjects engaged in two separate, and blinded experimental conditions: a placebo condition and an NAC condition. Each experimental condition consisted of a day of baseline testing, a treatment period lasting between 8 days, and one or two post-treatment testing sessions. In total, subjects were asked to report to the laboratory on eight separate occasions. A visual representation of the study design can be seen below:





Day 1a and 1b: The initial baseline visit included completion of informed consent, and general training questionnaire. Additionally, subjects underwent baseline measurements of height, weight, body composition, blood collection, and a measure of total hemoglobin mass. Following these measurements on Day 1a, subjects were randomly assigned to counterbalanced groups and completed 8 days of blinded supplementation consisting of either NAC or placebo. Day 1b concluded with subjects beginning supplementation of the crossover treatment.

Day 9a and 9b: Subjects completed a post-supplementation test of total hemoglobin mass.

Day 10a and 10b: Subjects completed post-supplementation measures of height, weight, and body composition. After a blood draw, participants entered a normobaric hypoxic chamber for 6 hours and provided a blood sample at 3 additional time points (2,4, and 6 hours). Arterial oxyhemoglobin saturation and heart rate data were collected for the duration of the hypoxic session. Following the completion of Day 10a and 10b, subjects engaged in a three-week washout period to eliminate any residual effects from the initial supplementation period.

Supplementation: Subjects participating in the NAC group ingested 1800 mg/day in the form of three, 600mg doses taken at 8am, 1pm, and 6pm – or with breakfast, lunch and dinner. Subjects participating in the placebo group ingested capsules of sugar free gelatin in the same amount and

on the same time scale. The supplementation period lasted eight days as previous literature has shown a significant rise in EPO levels in normoxia following eight days of NAC supplementation (Zembron-Lacny et al. 2009). Subjects were asked to keep a supplementation log to ensure compliance and track any missed doses. A three-week washout period was used between supplemental periods to ensure that there were no residual effects on future blood samples (Zembron-Lacny et al. 2009).

Hypoxic Exposure: Up to 4 subjects at a time were exposed to six hours of normobaric hypoxia using a Colorado Altitude Training Systems altitude chamber (CAT-430, Colorado Altitude Training Systems, Boulder, CO). To simulate hypoxia, a nitrogen generator was used to manipulate the atmospheric composition of the chamber to decrease the oxygen partial pressure within the tent to 15.8%; the equivalent to an altitude of 2500m / 8000 ft. To ensure that ambient CO₂ levels within the chamber were maintained, air was drawn out of the chamber, through a CO₂ scrubber, and then recirculated back into the chamber. Subjects were free to stand and move about the chamber as needed and fans were placed in the tent to help the subjects stay cool. Subjects were given lawn chairs for seating, had access to food and drink, and were free to engage in quiet activities in the chamber, including reading, video watching, internet, etc. Subjects were asked to void prior to entering the chamber but were asked to wear a face mask attached to a reservoir of 15.8% oxygen if they needed to leave the chamber.

SPECIFIC MEASURES

Height and weight: Height was measured using a stadiometer while weight was recorded using an electric scale.

Body Composition: Body composition was recorded using a bioelectrical impedance device. Subjects stood on an electrode imbedded scale while holding electrode impeded handles. An unnoticeable electric current was briefly sent through the subject from which, impedance was measured, and body composition estimated.

Blood sampling: For each blood sample, approximately 2 – 5 ml was taken via a routine venipuncture procedure from an antecubital vein using a 22g or smaller needle and a clot activator treated (red top) vacutainer for serum measures and an EDTA treated (purple top) vacutainer for reticulocyte measures. Every effort was made to ensure that blood draws occur at the same time during every visit.

Blood processing: Following manufacturer's instructions, red top vacutainers (for serum samples) were inverted 5 times, allowed 30 minutes to fully clot, then centrifuged for 10 minutes at 3000 RPM and 4 degrees C. Purple top vacutainers (for plasma samples) were inverted 8-10 times and centrifuged for 10 minutes at 3000 RPM and 4 degrees C. Serum or plasma was aliquoted, labeled, and frozen at -40 degrees C until analyzed. EPO concentration was determined by enzyme-linked immunosorbent assay (ELISA; Human Erythropoietin ELISA Kit ab11952, abcam, Cambridge, MA, USA; sensitivity 0.4 mIU/ml; detection range 1.6 – 100 mIU/ml) with samples analyzed according to manufacturer's instructions in triplicate using a Powerwave XS™ spectrophotometer (Bio-Tek Instruments, Winooski, VT).

Total hemoglobin mass: A 2-minute CO rebreathing method, as described by Gore (Gore et al., 2006) was used to determine total hemoglobin mass. In brief, subjects inhaled a 1 ml/kg bolus of 99.5% CO, which was rebreathed for two minutes from a homemade spirometer connected to a 5L anesthesia bag filled with 100% O₂. Finger prick 100 µL blood samples were collected pre-rebreathe and 6 and 8 minutes after the beginning of the rebreathe. These samples were analyzed (minimum in triplicate) for carboxyhemoglobin percentage (%HbCO) and hemoglobin concentration using a blood gas analyzer (OSM3, Radiometer, Copenhagen). Measures of end-tidal CO (Fluke model CO-220, Canada) at minute 5 after the start of the rebreathe, the volume of the spirometer post-rebreathe, and CO content of the spirometer post-rebreathe were determined. Assuming an alveolar ventilation of 15 L/min post-rebreathe and a residual volume of 1.5 L, total hemoglobin mass was calculated using the equation of Gore et al. (Gore et al., 2013).

Arterial Oxyhemoglobin Saturation: A fingertip pulse oximeter (Masimo Radical 7, Irvine, CA) was used to determine arterial oxyhemoglobin saturation in addition to heart rate every 2 hours while subjects were in the chamber.

Treatment of Data: A 2 x 4 repeated measures ANOVA and Tukey's post-hoc tests were used to assess differences between EPO responses, with treatment group (Placebo, NAC) and hypoxic exposure time (0, 2, 4, 6 hours) as within groups independent variables. Additionally, paired student's t-tests with a Bonferoni adjustment for multiple comparisons were used to determine differences between baseline and pre-hypoxic exposure timepoints in measures of serum EPO and total hemoglobin mass.

A power analysis used in previous literature (Hildebrandt et al, 2002) indicated that approximately 12 subjects would provide adequate statistical power (i.e. $1 - \beta > 0.80$) to show significant differences at the $p < 0.05$ level. All statistical measures and tests were conducted using Rstudio software.

CHAPTER 4

RESULTS

Subjects: A total of 16 subjects were recruited for the study. Six subjects dropped out of the study, all due to time constraints, leaving a total of 10 subjects for analysis. Subject characteristics are displayed below in Table 1. All subjects met the inclusion criteria for the study.

Table 1. Subject Characteristics

Height (m)	1.78 \pm 0.03
Weight (kg)	70.1 \pm 4.4
Body Fat (%)	10.9 \pm 2.2
Age (years)	26.3 \pm 4.0

Values are mean \pm SD, N=10

Hypoxic exposure:

The fractional oxygen content of the tent remained stable throughout all trials and there was no significant difference between the PLA and NAC conditions.

Supplementation:

Eight days of NAC supplementation prior to acute hypoxic exposure did not significantly alter the serum EPO, hematocrit, or Hb mass in elite endurance athletes when compared with the placebo condition as seen in Table 2 below:

Table 2. Supplementation Blood Data

Condition	PLA Base	PLA Pre	P-Value	NAC Base	NAC Pre	P-Value
Hbmas (g/kg)	15.75 ± 0.97	16.01 ± 0.68	0.38	15.58 ± 0.72	15.84 ± 0.97	0.24
Hematocrit (%)	46.2 ± 1.6	46.6 ± 1.9	0.57	45.8 ± 2.7	46.4 ± 2.2	0.49
Serum EPO (IU/L)	10.4 ± 2.9	9.9 ± 1.5	0.52	10.9 ± 2.5	10.2 ± 2.0	0.23

Values are mean ± SD, N = 10

Hbmass - total hemoglobin mass; Serum EPO - erythropoietin; PLA - placebo; NAC-n-acetyl cysteine; Base - baseline, prior to supplementation; Pre - pre-tent entry after 8 days of supplementation.

Hypoxia Exposure Data:

Hypoxic chamber data collected within each condition can be seen in table 3 below:

Table 3. Chamber Exposure Data

Placebo Condition				
Measures	0hr	2hr	4hr	6hr
Heart Rate (bpm)	60.9 ± 7.6	60.7 ± 7.2	60.6 ± 7.3	62.9 ± 5.3
Oxygen Saturation (%)	95.3 ± 1.2	95.2 ± 1.1	95.6 ± 1.7	95.2 ± 1.3
Serum EPO (IU/L)	9.93 ± 1.5	10.8 ± 2.3*	13.1 ± 2.9*	13.1 ± 3.1*
Serum EPO Δ (%)	-	9.3 ± 15.2	32.8 ± 25.0	32.3 ± 23.6
NAC Condition				
Heart Rate (bpm)	64.3 ± 6.4	68.5 ± 5.1	63.8 ± 6.0	64.8 ± 5.4
Oxygen Saturation (%)	95.0 ± 1.6	94.8 ± 1.8	95.4 ± 1.5	95.3 ± 1.1
Serum EPO (IU/L)	10.2 ± 2.0	12.5 ± 4.0*	12.6 ± 2.9*	13.4 ± 2.3*
Serum EPO Δ (%)	-	22.8 ± 19.1	24.1 ± 14.0	31.4 ± 20.9

Values are mean ± SD, N = 10

EPO - erythropoietin; EPO Δ – percent change in serum EPO from 0h

* = significantly different from 0hr, within condition

Heart rate and SaO₂ were not different at any time point, within either condition. Serum EPO was significantly increased from 0hr (tent entry) at 2h, 4h, and 6h in both conditions; however, the magnitude of increase was not different between conditions.

CHAPTER 5

DISCUSSION

The purpose of this study was to assess the effect of 8 days of NAC supplementation on serum EPO production in response to acute hypoxic exposure in trained endurance athletes. Our primary finding is that 8 days of NAC supplementation prior to acute hypoxic exposure has no significant impact on serum EPO concentration or erythropoiesis. The myriad of factors known to affect the oxygen cascade, taken in concert with the known individual variance in EPO response, suggest the existence of confounding factors beyond the relationship of antioxidant status and HIF-1 α stabilization regarding EPO production.

Trained endurance athletes utilize hypoxic training to increase EPO production and ultimately total hemoglobin mass. Since it has been established that the physiological response to hypoxia is proportional to hypoxic stress, one strategy that trained endurance athletes use to increase EPO and total Hb mass is to increase the hypoxic dose by living and training at higher altitudes (Eckardt et al. 1989). However, there are outcomes associated with living at altitudes above ~2500m which are ultimately negative for exercise performance (Chapman et al, 2016). Previous studies indicated that supplementation with NAC prior to acute hypoxic exposure might work to stabilize HIF1- α independent of hypoxia (Hildebrandt 2002; Zembron-Lacny 2009) and it was hypothesized that a pre-hypoxic, NAC supplementation period might allow for trained endurance athletes to maximize EPO response without increasing hypoxic dose. With that being said, despite following a nearly identical supplementation protocol as seen in Zembron-Lacny et al. 2009 and Hildebrandt et al. 2002, inter-group differences in mean EPO changes in response to the supplementation periods and subsequent acute hypoxic exposures were not found to be statistically significant and displayed very large standard deviation values. Further, intra-group

mean EPO changes in response to acute hypoxia were statistically significant. Taken together, the data suggest that, while each supplementation group had the expected EPO response to acute hypoxia, this response was not significantly different between these groups.

When considering the large standard deviations seen in the group mean data, it may be that they are reflective of the known individualized response to hypoxia within subjects (Chapman 1998). It was assumed that intra-individual serum EPO measures would yield consistent results across similar environmental conditions. Subject serum EPO response data, however, suggests that intra-individual EPO response may actually be more variable than previously thought as subjects experienced an average of 48% variation in EPO response across similar environmental conditions. These data appear to be supported by the conclusions drawn from a similar study which showed an average variation of 54% intra-individual EPO response (Baranauskas et al, HAMB, in press). This natural volatility in intra-subject EPO response may have concealed any effect that the NAC supplementation period may have had on erythropoiesis and may have contributed to the large standard deviations seen in group mean data.

Limitations:

A power analysis using previous literature (Hildebrandt et al, 2002) indicated that approximately 12 subjects would provide adequate statistical power (i.e. $1 - \beta > 0.80$) to show significant differences at the $p < 0.05$ level. The present study recruited 16 individuals but only 10 individuals finished the study. A power analysis completed after $N=10$ indicated that > 100 subjects would need to be recruited with the observed treatment effect to see statistical significance, so a decision was made to terminate the experiment.

A hypoxic inspirate of 15.8%, simulating 2500m, was chosen for this study, as this is a common altitude utilized by elite endurance athletes when engaged in altitude training. It is

possible that with a relatively short exposure duration of 6hr, a larger hypoxic stimulus was needed to observe differences in EPO between placebo and NAC conditions.

Zembron-Lacny et al 2009 and Hildebrandt et al 2002 served as the roadmap for this project and both studies measured blood thiol levels both before and after the eight-day supplementation period. In hindsight, this data may have given insight into the effect that the supplementation period may have had on subject antioxidant status and may have given insight into the effectiveness of the NAC dose and the subsequently measured serum EPO response.

In conclusion, eight days of supplementation with 1800mg of NAC did not significantly alter production of serum EPO after six hours of exposure to a simulated altitude of 2500m in elite endurance athletes. Due to the variance in hematological response experienced by the participants in the present study, future studies into the subject matter may look to include stricter subject controls in an effort control the large intra-subject variation in EPO response to acute hypoxia.

REFERENCES

- Aguiló, A., Tauler, P., Fuentespina, E., Villa, G., Córdova, A., Tur, J. A., & Pons, A. (2004). Antioxidant diet supplementation influences blood iron status in endurance athletes. *International journal of sport nutrition and exercise metabolism*, 14(2), 147-160.
- Arendshorst, WJ, and LG Navar. 1993. "Renal Circulation and Glomerular Hemodynamics." In *Diseases of the Kidney*, 1:65–117. Little, Brown Boston.
- Bärtsch, P, and B Saltin. 2008. "General Introduction to Altitude Adaptation and Mountain Sickness." *Scandinavian Journal of Medicine & Science in Sports* 18 (s1): 1–10.
- Bauer, C. 1988. "Metabolic Events That May Activate Erythropoietin Production in the Hypoxic Kidney." In *Oxygen Sensing in Tissues*, 93–101. Springer.
- Bauer, Christian, and Armin Kurtz. 1989. "Oxygen Sensing in the Kidney and Its Relation to Erythropoietin Production." *Annual Review of Physiology* 51: 845–856.
- Buskirk, ER, J Kollias, RF Akers, EK Prokop, and EP Reategui. 1967. "Maximal Performance at Altitude and on Return from Altitude in Conditioned Runners." *J Appl Physiol* 23 (2): 259–266.
- Chandel, Navdeep S, David S McClintock, Carlos E Feliciano, Teresa M Wood, J Andres Melendez, Ana M Rodriguez, and Paul T Schumacker. 2000. "Reactive Oxygen Species Generated at Mitochondrial Complex III Stabilize Hypoxia-Inducible Factor-1 α during Hypoxia A MECHANISM OF O₂ SENSING." *Journal of Biological Chemistry* 275 (33): 25130–25138.
- Chapman, Robert F, Stray-Gundersen J, Levine BD. Erythropoietin production at altitude in elite endurance athletes is not mediated by peripheral chemoresponsiveness. *Journal of Science and Medicine in Sport*. 13: 624-629, 2010.
- Chapman, R. F., Karlsen, T., Ge, R. L., Stray-Gundersen, J., & Levine, B. D. (2016). Living altitude influences endurance exercise performance change over time at altitude. *Journal of Applied Physiology*, 120(10), 1151-1158.
- Chapman, Robert F, Trine Karlsen, Geir K Resaland, R-L Ge, Matthew P Harber, Sarah Witkowski, James Stray-Gundersen, and Benjamin D Levine. 2014. "Defining the 'dose' of Altitude Training: How High to Live for Optimal Sea Level Performance Enhancement." *Journal of Applied Physiology* 116 (6): 595–603.
- Chapman, Robert F, James Stray-Gundersen, and Benjamin D Levine. 1998. "Individual Variation in Response to Altitude Training." *Journal of Applied Physiology* 85 (4): 1448–1456.
- Duke, Joseph W, Robert F Chapman, and Benjamin D Levine. 2012. "Live-High Train-Low Altitude Training on Maximal Oxygen Consumption in Athletes: A Systematic Review and Meta-Analysis: A Commentary." *International Journal of Sports Science & Coaching* 7 (1): 15–19.
- Duncan Jr, Leroy E, Grant W Liddle, Frederic C Bartter, and Katherin Buck. 1956. "The Effect of Changes in Body Sodium on Extracellular Fluid Volume and Aldosterone and Sodium Excretion by Normal and Edematous Men." *Journal of Clinical Investigation* 35 (11): 1299.
- Eckardt, Kai-Uwe, Urs Boutellier, Armin Kurtz, Michael Schopen, Erwin A Koller, and Christian Bauer. 1989. "Rate of Erythropoietin Formation in Humans in Response to Acute Hypobaric Hypoxia." *Journal of Applied Physiology* 66 (4): 1785–1788.

- Feraillie, Eric, and Alain Doucet. 2001. "Sodium-Potassium-Adenosinetriphosphatase-Dependent Sodium Transport in the Kidney: Hormonal Control." *Physiological Reviews* 81 (1): 345–418.
- Ferrara, Napoleone, Hans-Peter Gerber, and Jennifer LeCouter. 2003. "The Biology of VEGF and Its Receptors." *Nature Medicine* 9 (6): 669–676.
- Fisher, James W. 1983. "Control of Erythropoietin Production." *Experimental Biology and Medicine* 173 (3): 289–305.
- Garvican, L, D Martin, M Quod, B Stephens, A Sassi, and C Gore. 2012. "Time Course of the Hemoglobin Mass Response to Natural Altitude Training in Elite Endurance Cyclists." *Scandinavian Journal of Medicine & Science in Sports* 22 (1): 95–103.
- Grupp, I, G Grupp, JC Holmes, and NO Fowler. 1972. "Regional Blood Flow in Anemia." *Journal of Applied Physiology* 33 (4): 456–461.
- Hackett, Peter H, and Robert C Roach. 2001. "High-Altitude Illness." *New England Journal of Medicine* 345 (2): 107–114.
- Harms, CRAIG A, and JOEL M Stager. 1995. "Low Chemoresponsiveness and Inadequate Hyperventilation Contribute to Exercise-Induced Hypoxemia." *Journal of Applied Physiology* 79 (2): 575–580.
- Hildebrandt, Wulf, Steve Alexander, Peter Bärtzsch, and Wulf Dröge. 2002. "Effect of N-Acetyl-Cysteine on the Hypoxic Ventilatory Response and Erythropoietin Production: Linkage between Plasma Thiol Redox State and O₂ Chemosensitivity." *Blood* 99 (5): 1552–1555.
- Honig, ARNOLD. 1983. "Role of the Arterial Chemoreceptors in the Reflex Control of Renal Function and Body Fluid Volumes in Acute Arterial Hypoxia." *Physiology of the Peripheral Arterial Chemoreceptors. Elsevier, Amsterdam*, 395–429.
- . 1989. "Peripheral Arterial Chemoreceptors and Reflex Control of Sodium and Water Homeostasis." *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 257 (6): R1282–R1302.
- Huang, L Eric, Zoltan Arany, David M Livingston, and H Franklin Bunn. 1996. "Activation of Hypoxia-Inducible Transcription Factor Depends Primarily upon Redox-Sensitive Stabilization of Its α Subunit." *Journal of Biological Chemistry* 271 (50): 32253–32259.
- Jaakkola, Panu, David R Mole, Ya-Min Tian, Michael I Wilson, Janine Gielbert, Simon J Gaskell, Alexander von Kriegsheim, et al. 2001. "Targeting of HIF- α to the von Hippel-Lindau Ubiquitylation Complex by O₂-Regulated Prolyl Hydroxylation." *Science* 292 (5516): 468–472.
- Koh, Mei Yee, Taly R Spivak-Kroizman, and Garth Powis. 2008. "HIF-1 Regulation: Not so Easy Come, Easy Go." *Trends in Biochemical Sciences* 33 (11): 526–534.
- Lassen, Niels A, Ole Munck, and Jørn Hess Thaysen. 1961. "Oxygen Consumption and Sodium Reabsorption in the Kidney." *Acta Physiologica Scandinavica* 51 (4): 371–384.
- Lee, Ji-Won, Seong-Hui Bae, Joo-Won Jeong, Se-Hee Kim, and Kyu-Won Kim. 2004. "Hypoxia-Inducible Factor (HIF-1)[α]: Its Protein Stability and Biological Functions." *Experimental & Molecular Medicine* 36 (1): 1.
- Levine, BD, and J Stray-Gundersen. 1992. "A Practical Approach to Altitude Training." *International Journal of Sports Medicine* 13 (S 1): S209–S212.
- Levine, Benjamin D, and James Stray-Gundersen. 1997. "'Living High-Training Low': Effect of Moderate-Altitude Acclimatization with Low-Altitude Training on Performance." *Journal of Applied Physiology* 83 (1): 102–112.

- . 2006. “Dose-Response of Altitude Training: How Much Altitude Is Enough?” In *Hypoxia and Exercise*, 233–247. Springer.
- Li, Zaibo, Dakun Wang, Edward M Messing, and Guan Wu. 2005. “VHL Protein-Interacting Deubiquitinating Enzyme 2 Deubiquitinates and Stabilizes HIF-1 α .” *EMBO Reports* 6 (4): 373–378.
- Maret, W. (2000). The function of zinc metallothionein: a link between cellular zinc and redox state. *The Journal of nutrition*, 130(5), 1455S-1458S.
- Naeije, Robert. 2010. “Physiological Adaptation of the Cardiovascular System to High Altitude.” *Progress in Cardiovascular Diseases* 52 (6): 456–466.
- Niess, Andreas Michael, Elvira Fehrenbach, Günther Strobel, Kai Roecker, Elisabeth Marion Schneider, Julia Buergler, Simone Fuss, Rainer Lehmann, Hinnak Northoff, and HANS-HERMANN Dickhuth. 2003. “Evaluation of Stress Responses to Interval Training at Low and Moderate Altitudes.” *Medicine and Science in Sports and Exercise* 35 (2): 263–269.
- Ohh, Michael, Cheol Won Park, Mircea Ivan, Michael A Hoffman, Tae-You Kim, L Eric Huang, Nikola Pavletich, Vincent Chau, and William G Kaelin. 2000. “Ubiquitination of Hypoxia-Inducible Factor Requires Direct Binding to the β -Domain of the von Hippel–Lindau Protein.” *Nature Cell Biology* 2 (7): 423–427.
- Rhodes, Kate, and Andrea Braakhuis. 2017. “Performance and Side Effects of Supplementation with N-Acetylcysteine: A Systematic Review and Meta-Analysis.” *Sports Medicine*, 1–18.
- Roach, RC, D MAES, D SANDOVAL, RA ROBERGS, M ICENOGLE, H HINGHOFFER-SZALKAY, D Lium, and JA Loeppky. 2000. “Exercise Exacerbates Acute Mountain Sickness at Simulated High Altitude.” *J. Appl. Physiol* 88: 581–585.
- Schneider, MICHAEL, DIRKE Bernasch, JORN Weymann, ROLF Holle, and P Bartsch. 2002. “Acute Mountain Sickness: Influence of Susceptibility, Preexposure, and Ascent Rate.” *Medicine and Science in Sports and Exercise* 34 (12): 1886–1891.
- Simon, M Celeste. 2006. “Mitochondrial Reactive Oxygen Species Are Required for Hypoxic HIF α Stabilization.” In *Hypoxia and Exercise*, 165–170. Springer.
- Tajima, Masamichi, Yukiko Kurashima, Kenji Sugiyama, Tsutomu Ogura, and Hiroshi Sakagami. 2009. “The Redox State of Glutathione Regulates the Hypoxic Induction of HIF-1.” *European Journal of Pharmacology* 606 (1): 45–49.
- Warren, James V, Arthur J Merrill, and Eugene A Stead Jr. 1943. “The Role of the Extracellular Fluid in the Maintenance of a Normal Plasma Volume.” *Journal of Clinical Investigation* 22 (5): 635.
- Wehrlin, Jon Peter, Peter Zuest, Jostein Hallén, and Bernard Marti. 2006. “Live High-Train Low for 24 Days Increases Hemoglobin Mass and Red Cell Volume in Elite Endurance Athletes.” *Journal of Applied Physiology* 100 (6): 1938–1945.
- Wilber, Randall L. 2007. “Application of Altitude/hypoxic Training by Elite Athletes.” *Medicine and Science in Sports and Exercise* 39 (9): 1610–1624.
- Wisnibaugh, Paul E, Josephine B Garst, Charles Hull, Richard J Freedman, Dean N Matthews, and Magdalena Hadady. 1972. “Renin, Aldosterone, Sodium and Hypertension.” *The American Journal of Medicine* 52 (2): 175–186.
- Zembron-Lacny, A, M Slowinska-Lisowska, Z Szygula, Z Witkowski, and K Szyszka. 2010. “Modulatory Effect of N-Acetylcysteine on pro-Antioxidant Status and Haematological Response in Healthy Men.” *Journal of Physiology and Biochemistry* 66 (1): 15–21.

Zembron-Lacny, Agnieszka, M Slowinska-Lisowska, Z Szygula, K Witkowski, and K Szyszka. 2009. "The Comparison of Antioxidant and Hematological Properties of N-Acetylcysteine and [Alpha]-Lipoic Acid in Physically Active Males." *Physiological Research* 58 (6): 855.

TYLER NOBLE - CV

EDUCATION

MS Indiana University - Bloomington, Exercise Physiology May 2021
Thesis: "N-Acetyl Cysteine Supplementation does not Augment Serum EPO
Response in Trained Endurance Athletes."
Advisor: Dr. Robert Chapman

BS Saginaw Valley State University, Exercise Science
Minored in Psychology

HONORS AND AWARDS

IU Bloomington School of Public Health Fellowship Grant 2016
Financial assistance to aid in the completion of an original research project

SVSU Dean's List	2012-14
Recognition of academic success	

RESEARCH EXPERIENCE

Master's Thesis, Indiana University, Bloomington, IN 2021
 Advisor: Dr. Robert Chapman

- Blood collection, processing, and storage
- ELISA to measure serum EPO concentrations
- Hypoxic chamber calibration and operation
- Study design and IRB submission
- Statistical analysis and inference of collected data

TEACHING EXPERIENCE

Saginaw Valley State University, University Center, MI January 2012 to May 2014

Teaching Assistant, Kinesiology

- Calibrated and operated exercise equipment in the human performance lab
- Taught select topics as designated by the lead instructor
- Proctored exams and lab assignments

PUBLICATIONS

Journal Publications

Noble, T.J., Chapman, R.F., “Marathon Specialization in Elites: A Head Start for Africans,” *International Journal of Sports Physiology and Performance*, vol. 13, no. 1, 2018, pp. 102-106.

Wilhite, D.P., Foss, J., Noble, T.J., Bergland, D., Stray-Gundersen, J., Chapman, R.F., “Hematological Response to Uncontrolled Use of Altitude Training by Elite Distance Runners,” *Medicine and Science in Sports and Exercise*, vol. 47, no. 5, 2015, pp. 248-248.

Conference Abstracts

Noble, T.J., and Chapman, R.F., “Run Performance is the Primary Factor in Determining Olympic Distance Triathlon Performance,” *Proceedings of ACSM Annual Meeting*, June 2nd, 2018, *Medicine & Science in Sports & Exercise* 50 (5s), pp. 776

Noble, T.J., and Chapman, R.F., “The Retention Potential of the 2016 US Olympic Track and Field Team,” *Proceedings of ACSM Annual Meeting*, June 2nd, 2017, *Medicine & Science in Sports & Exercise* 49 (5s), pp. 736.

Noble, T.J., and Chapman, R.F., “Elite African Male Marathoners Start and Peak at a Younger Age than Non-Africans,” *Proceedings of ACSM Annual Meeting*, June 3rd, 2016, *Medicine & Science in Sports & Exercise* 48 (5s), pp. 851.

PRESENTATIONS AND INVITED LECTURES

Workshop, “Talent Identification Practices in 2020”, United States Olympic and Paralympic Committee Data and Analytics Conference, 11/4/2020.

Workshop, “Predicting Performance and Determining Program Effectiveness”, United States Olympic and Paralympic Committee Data and Analytics Conference, 10/3/2018.

PROFESSIONAL AFFILIATIONS

USA Track & Field, Sport Science & Data Analytics Manager, June 2018 - Present

- Leverage a network of sport science professionals to positively impact athlete performance.
- Forecast athlete performance to determine resource allocation
- International travel to over 20 countries for national team support

USA Track & Field, Sport Medicine Coordinator, May 2016 – June 2018

- Coordinate acute and chronic athlete care
- Coordinate and manage medical care at national team events on the international stage

USA Triathlon, National Office Intern, Fall 2014

- Worked with every department at the national office with an emphasis on elite athlete development.
- Development of sound talent identification practices to grow the elite athlete pool

USA Track & Field, High performance & Coaching Education Intern, Summer 2014

- Coordinated and managed coaching education Level I and Level II courses
- Assisted the Chief of High Performance in the day to day departmental operations

PROFESSIONAL CERTIFICATIONS

Level II Endurance Specialist Certification, USA Track & Field, Summer 2012

Level II Sprints, Hurdles, and Relays Certification, USA Track & Field, Summer 2013

Endurance Specialist Certification, USTFCCCA, Summer 2013

ADDITIONAL SKILLS

Programming: RStudio, Python, SQL

Applications: Microsoft Office, Google Suite

Travel: International Travel to 23 different countries as of May 2021

REFERENCES

Available upon request